1544–1545 *Nucleic Acids Research, 1998, Vol. 26, No. 6*

In vivo mapping of nucleosomes using psoralen–DNA crosslinking and primer extension

Ralf Erik Wellinger and José M. Sogo*

Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

Received December 16, 1997; Revised and Accepted February 2, 1998

ABSTRACT

By the use of psoralen crosslinking and primer extension, a method was developed which allows the analysis of chromatin structure *in vivo*. Using a yeast minichromosome, >9 nucleosomes were mapped with a resolution of at least ±30 bp.

In the nucleus of eukaryotic cells, DNA is packaged into a nucleoprotein complex known as chromatin. This complex provides the compaction and structural organisation of the DNA for processes such as replication, transcription, recombination and repair. A great deal of recent work has been directed towards understanding the impact of the basic repeating subunit of chromatin, the 'nucleosome' on these processes. Over the last decades several techniques have been developed to characterise chromatin structure including defining the positioning of nucleosomes to specific DNA sequences. Typically, nucleosome positions were defined by enzymatic (e.g. MNase or DNase I) and/or chemical treatment of chromatin (e.g. hydroxyl radical) (1). However, most of these techniques require the isolation of nuclei prior to nucleosomal analysis, such that the isolation *per se* may somewhat distort the *in vivo* chromatin structure. We therefore developed a direct biochemical approach that allows the mapping of nucleosomes *in vivo*. Since psoralen derivatives easily penetrate cellular membranes of living cells (2), we anticipate that the technique described here is applicable to most cell types including plant cells (3). The developed assay is extrapolable to soluble chromatin or isolated nuclei, however, intact vertebrate cells will be the most exciting substrate.

The psoralen crosslinking technique has successfully been proven to be a useful tool for the study of chromatin structure (2–6). Psoralens efficiently intercalate in helical DNA and upon irradiation with ultraviolet (UV) light (366 nm) form covalent crosslinks between pyrimidines of opposite strands. However, in chromatin, psoralen crosslinking occurs preferentially within the linker DNA whereas the nucleosomal DNA is protected against crosslinking (Fig. 1). Trimethylpsoralen (TMP) is the most commonly used psoralen for chromatin studies. It has been shown that even extensive TMP crosslinking of chromosomal DNA, performed under nearly saturating conditions, neither destroys nor disturbs the nucleosomal and higher order structure of chromatin (6,7).

We have developed a technique, utilising the advantages of psoralen crosslinking and primer extension to accurately map the positions of nucleosomes in the TRURAP plasmid (8). This has been previously well characterised by alternative methods, allowing us to directly compare the published (8,9) and the newly generated data. The developed technique is presented in detail in Figure 1. Analysis of the primer extension products on an alkaline agarose gel (Fig. 2A and B) showed a premature blockage of the reaction, consistent with a block of the *Taq* polymerase at psoralen crosslinks. In naked DNA the signal reflects the psoralen sequence specificity (1). With increased crosslinking this sequence specificity is more pronounced and appears as 'hot spots'. However, the obtained crosslinking pattern of naked DNA differs from those generated for DNA crosslinked in chromatin. The possible appearance of a non-crosslinkable DNA sequence is greatly reduced in TRURAP, since we calculated potential crosslink sites at a minimum of 6 bp intervals. In contrast, specific reaction products were obtained for chromatin. The specific pattern seen in chromatin reflects an inhibition of crosslinking in the DNA wrapped around the histone octamers (compare positioned nucleosomes 1–6 in the URA3 gene) which results in a specific crosslinking of the linker DNA. All nucleosomes in the URA3 gene are easily detectable indicating that psoralen can also penetrate between tightly packaged nucleosomes. For visual comparison an example of 'classical' MNase pattern obtained by indirect end labelling analysis of digested nuclei isolated from the same strain is presented in Figure 2C. Note that the location of linkers detected by both techniques is nearly identical (compare Fig. 2B, lane 9 with Fig. 2C, lane 16). Statistical analysis of the linker positions shown in Figure 2D gave values very close to those previously published by MNase analysis (8,9). Since the weak, unspecific signals present in the non-crosslinked DNA were not detectable in chromatin they did not interfere with the interpretation of the results.

FTY23 was grown as described (8). About 1×10^9 cells were concentrated in 1.5 ml TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA), transferred to trays to form thin layers (2 mm) and 75 µl TMP [(200 µg/ml): 4,5′,8-trimethylpsoralen (Sigma) in ethanol] was added. After 5 min incubation, cells were irradiated on ice with various UV-doses (Sylvania G15T8 black light blue lamps, 366 nm at 30 J/m^2/s) at a distance of 2 cm. DNA was isolated using Qiagen Genomic tip G/20 and dissolved in 100 µl TE (~150 ng/µl DNA). As control, DNA from 1×10^9 cells was isolated as described, dissolved in 300 µl TE, transferred to a tray (2.5 mm layer), 15 µl TMP (200 µg/ml) was added and the sample was incubated for 5 min on ice. Irradiation was performed at a distance of 16 cm (15 J/m^2/s). Control DNA in 150 mM NaCl was extracted three times with 1 vol of dichloromethane/isoamylalcohol (24/1), ethanol precipitated and dissolved in 100 µl TE (150 ng/µl DNA). From both samples, ~3 µg DNA were restricted with *Eco*RI. The overhanging 5′-end was filled in using Klenow DNA polymerase (2 U, Böhringer MA) and nucleotides (0.1 mM final conc., Pharmacia). The DNA was purifed using columns (Böhringer MA, Cat. No. 1732668) and eluted with 100 µl TE. To 44 µl of the blunt ended DNA, 5 µl 10× exonuclease buffer (670 mM glycine KOH

*To whom correspondence should be addressed. Tel: +41 1 633 3342; Fax: +41 1 633 1069; Email: sogo@cell.biol.ethz.ch

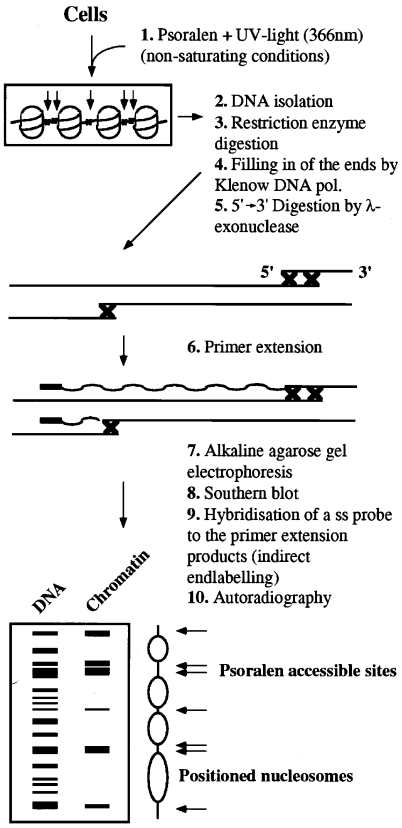

Figure 1. Schematic representation of nucleosome mapping by psoralen crosslinking and primer extension.

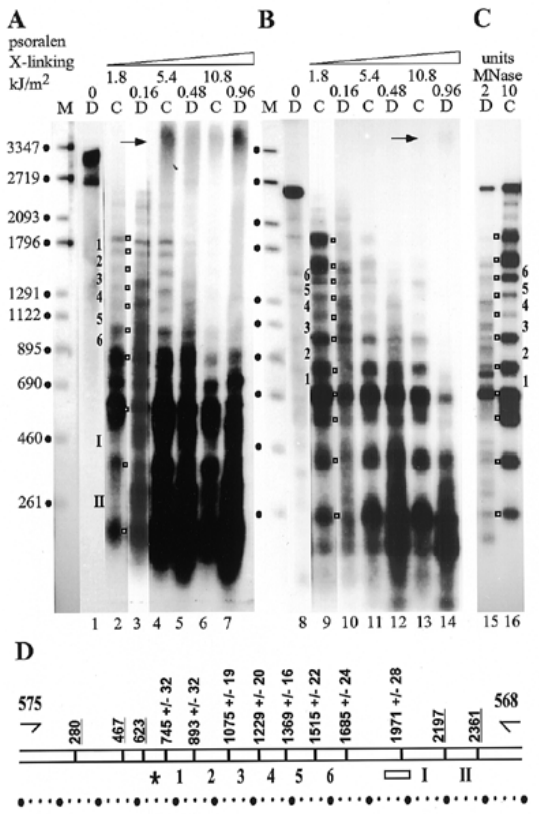

Figure 2. Analysis of the YRpTRURAP nucleosome structure on the bottom and top strand by primer extension analysis of λ-exonuclease treated DNA. (**A**) Mapping of the bottom strand using primer 568. After primer extension [primer 568: 5′-CGGATCTCGCATTGATGAGGCAACGC-3′; 2539–2514 map units (MU) (12)], DNA was run in a 1.5% alkaline agarose gel, transferred to a nylon membrane (Pall B) and hybridised to a radioactively labelled, single-stranded probe matching 2379–2539 MU. The location of psoralen crosslinks in naked DNA (D) and chromosomal DNA (C) is shown. Numbers on the top indicate the applied UV-dose (366 nm, kJ/m^2). Size marker is shown (M). The arrow indicates highly crosslinked DNA which remains double stranded. Non-crosslinked DNA was also used as a control (lane 1). Crosslinking specifically obstructs the extension reaction (lanes 2–7) and increased crosslinking causes a premature blockage of the polymerase. The deduced location of linker DNA (open rectangles) and nucleosomes in the URA3 gene (from 1 to 6) is shown on the flanking sides of lane 2. (**B**) Mapping of the top strand using primer 575. Primer extension was performed using primer 575 [5′-GAGGGCCAAGAGGGAGGG-CATTGGTGAC-3′; 25–52 MU (9)]. Hybridisation was performed using a radioactively labelled, single-stranded probe matching 159–25 MU. Figure is labelled as in (A). (**C**) Mapping of the top strand by MNase digestion of nuclei and indirect end labelling. For technical details see (8,9). (**D**) Location of nucleosomes on TRURAP. Indicated are; location of primers 575 and 568 (halved arrows), linker DNA {bars, top numbers [open rectangles in (A) and (B) indicate the bands used for measurement]}, location of nucleosomes (bottom numbers), functional elements (star, promoter; open rectangle, origin of replication ARS1). Nucleosomes 1–6 and I, II are denoted below. Dots are in 200 and 50 bp intervals. The standard deviation was calculated for the position of bands present in both gels (the centre of each band was defined as linker position). Data represent the average of three independent experiments.

pH 9.4, 25 mM $MgCl_2$, 500 µg/ml BSA) and 1 µl λ-exonuclease (2.9 U, Gibco BRL) were added, incubated at 37°C for 30 min and purified by adding 1 µl Strataclean resin (Stratagene Cat. No. 400714). The resin was removed by centrifugation. Samples were adjusted by adding 1 vol of buffer (20 mM Tris–HCl, pH 6.1, 55 mM KCl, 4.3 mM $MgCl_2$). To 10 µl preadjusted DNA (~150 ng total DNA) 1 µl 10× PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 30 mM $MgCl_2$), 1 µl dNTP stock solution (5 mM/µl) 1 µl primer (0.1 pmol/µl) and 7 µl diluted *Taq* DNA polymerase (1 U, Perkin Elmer) were added. The samples were overlaid with 25 µl paraffin oil and subjected to 30 cycles of repeated denaturation at 95°C for 45 s, annealing at 65°C for 5 min and extension for 3 min at 72°C.

In summary, we can accurately map linker positions *in vivo* by psoralen crosslinking, obtaining very similar data to those published using isolated nuclei and micrococcal nuclease nucleosome mapping.

ACKNOWLEDGEMENTS

We like to thank Dr F.Thoma and M.Livingstone for providing a MNase nucleosome mapping of YRpTRURAP (Fig. 2C) and Drs R.Lucchini and K.Nightingale for critical reading of the manuscript.

REFERENCES

1 Lilley, D. M. J. and Dahlberg, J. E. (1992) *Methods Enzymol.*, **212**, 133–301.
2 Hanson, C., Shen, C. and Hearst, J. (1976) *Science*, **193**, 62–64.
3 Conconi, A., Sogo, J. M. and Ryan, C. A. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 5256–5260.
4 Sogo, J. M. and Thoma, F. (1989) *Methods Enzymol.*, **170**, 142–165.
5 Lucchini, R. and Sogo, J. M. (1998) In Paule, M. R. (ed), *RNA Polymerase I*, R. G. Landis Company, Austin, TX, USA. pp. 235–274.
6 Conconi, A., Losa, R., Koller, T. and Sogo, J. (1984) *J. Mol. Biol.*, **178**, 920–928.
7 Gale, J. M. and Smerdon, M. J. (1988) *Biochemistry*, **27**, 7197–7205.
8 Thoma, F. (1986) *J. Mol. Biol.* **190**, 177–190.
9 Tanaka, S., Livingstone, M. and Thoma, F. (1996) *J. Mol. Biol.*, **257**, 919–934.